Vector for Activating the Immune System Against Cells Associated to Papilloma Viruses or Sequences Thereof

The present invention relates to a vector suited for activating the immune system against cells associated to papilloma viruses and sequences thereof, respectively, a vaccination agent which contains such a vector and the use of both.

Papilloma viruses infect the epithelium of man and animal. Human papilloma viruses (HPVs) are found in benignant, e.g. warts, condylomas in the genital region, and malignant, e.g. carcinomas of skin and uterus, epithelial neoplasms. HPVs are also considered for the development of malignant tumors of the respiratory system. In addition, HPVs are considered to be at least jointly responsible for the development of squamous carcinomas of the lungs.

Papilloma viruses have an icosahedral capsid without coat which has a circular double-stranded DNA molecule of about 7900 bp. The capsid comprises a major capsid protein (L1) and a minor capsid protein (L2). The former is coded by the open reading frame L1 (L1-ORF) and the latter is coded by L2-ORF. L1 or L1 and L2 result *in vitro* in the formation of virus-like particles (VLPs).- The transformation ability of papilloma viruses is ascribed to the proteins E6 and E7. They are coded by E6-ORF and E7-ORF, respectively.

Many attempts have been made to stimulate the immune system over cells associated to papilloma viruses and sequences thereof, respectively. However, these attempts have not yet yielded satisfactory results.

Therefore, it is the object of the present invention to provide a product serving for activating the immune system to identify and eliminate cells, particularly tumor cells,

associated to papilloma viruses and sequences thereof, respectively.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a vector having a nucleic acid coding for a fusion polypeptide, the fusion polypeptide comprising a structural papilloma virus (poly)peptide and a non-transforming (poly)peptide coded by an early papilloma virus gene.

The expression "vector" comprises any vector which is suitable for gene transfer, i.e. the introduction of nucleic acids into cells. The vector may remain episomally within the cells or be integrated within the genome. Moreover, the vector may be a plasmid or virus vector. Examples of a virus vector are retroviral, adenovirus, vacciniavirus or adeno-associated virus (AAV) vectors, the latter being preferred. An AAV vector may be present in wild-type or modified form. It can also comprise only those sequences such as ITR sequences, that are necessary for its transduction ability. However, it can also be favorable for it to comprise additionally those sequences, such as rep sequences, which render possible for it the integration into chromosome 19. A virus vector can be present as viral particle or in the form of its nucleic acid. It is preferred for the virus vector to be replication-defective.

The expression "papilloma virus" comprises any papilloma viruses or sequences thereof, which can be associated with cells, particularly tumor cells. In particular, HPVs and more particularly "high risk" HPVs, such as HPV 16, 18, 33, 35 and 45, may be concerned.

The expression "nucleic acid" comprises any nucleic acid such as DNA and/or RNA, which codes for a fusion polypeptide comprising a structural papilloma virus (poly)peptide and a non-transforming (poly)peptide coded by an early papilloma

virus gene. It is favorable for the nucleic acid to be expressible. It is particularly favorable for it to be controlled by a constitutive or inducible promoter such as a tissue-specific or tumor-specific promoter.

The expression "structural papilloma virus (poly)peptide" comprises any peptide and polypeptide, respectively, of a papilloma virus, which is at least jointly responsible for the structure of the papilloma virus. In particular, such a (poly)peptide is coded by L1-ORF or L2-ORF of a papilloma virus and by part thereof, respectively. A (poly)peptide which can be present as VLP is particularly preferred.

The expression "a non-transforming (poly)peptide encoded by an early papilloma virus gene" comprises any peptide and polypeptide, respectively, which is coded by an early papilloma virus gene, particularly E1-, E2-, E4-, E5-, E6- or E7-ORF and by part thereof, respectively, and is non-transforming. The expression "non-transforming" refers to the fact that the (poly)peptide has no transformation ability by nature or by intervention. A preferred (poly)peptide is coded by E6-ORF or E7-ORF of a papilloma virus and by part thereof, respectively.

The expression "fusion polypeptide" refers to the fact that the structural papilloma virus (poly)peptide and the non-transforming (poly)peptide coded by an early papilloma virus gene can be present in any combination within the fusion polypeptide. The individual (poly)peptides may also originate from different papilloma viruses. The C terminus of the structural (poly)peptides is preferably connected with the N terminus of the non-transforming (poly)peptide. In addition, it may be advantageous for the non-transforming (poly)peptide to be localized within the structural (poly)peptide. A preferred fusion polypeptide comprises a (poly)peptide coded by HPV 16L1-ORF and a (poly)peptide coded by HPV 16 E6-ORF and E7-ORF, respectively. Furthermore, a fusion polypeptide is preferred which comprises a (poly)peptide coded by HPV 18 L1-ORF and a

(poly)peptide coded by HPV 18 E6-ORF and E7-ORF, respectively.

Common methods can be carried out for the preparation of an above vector. For example, an AAV vector can be prepared as virus particle as follows: The 5' end of the HPV 16 E6-ORF is ligated to the 3' end of the HPV 16 L1-ORF. Part of the E6-ORF had been deleted beforehand, so that the transforming properties of E6 were destroyed. The DNA fragment L1-ORF-E6-ORF is inserted in an AAV vector which contains the 5'-ITR and 3'-ITR sequences of AAV but not the sequences coding for the AAV Rep and AAV Cap proteins. The insertion is made between the two ITR sequences. The DNA fragment L1-ORF-E6-ORF is controlled by a promoter heterologous with respect to AAV. The resulting AAV vector is transfected in cells, which express the AAV Rep and AAV-Cap proteins. Furthermore, the cells are infected with a helper virus, e.g. adenovirus, so that the AAV vector is obtained as viral particle.

The immune system can be activated with an above vector, to identify and eliminate cells, particularly tumor cells, associated to papilloma viruses and sequences thereof, respectively. This can be achieved prophylactically and in a treatment. For this purpose, cells of the particular organism, such as antigen-presenting cells, e.g. dendritic cells, B cells, macrophages and/or tumor cells and/or pre-tumor cells, associated to papilloma viruses and sequences thereof, respectively, are transduced with the vector. The transduction can be made by common methods. If the vector is available as virus particle, it will be favorable to infect the cells therewith. On the other hand, if it is available as nucleic acid, e.g. DNA, it will be advisable to transfect the cells therewith. Electroporation, lipofection and particle gun have to be mentioned as transfection techniques by way of example. The cells may be present in the organism. On the other hand, the cells to be transduced can also be isolated from the organism, be transduced outside the organism and then be returned to the organism again. Such cells are referred to as autologous cells. Moreover,

allogenic cells can also be used for the transduction regarding the organism. In this connection, it is favorable for these cells to belong to an HLA type corresponding to the organism. The person skilled in the art is familiar with processes of providing cells with a certain HLA type. In addition, it is favorable if, in an above process, the tumor cells or pre-tumor cells are inactivated before they are returned to the organism. For this purpose, common methods, such as irradiation, can be carried out.

Another subject matter of the present invention relates to a vaccination agent which comprises an above vector and common auxiliary substances, such as buffers, diluents, carriers, etc. It can be favorable for the vaccination agent to contain further substances which can activate the immune system, e.g. against tumor cells. Such substances can be particularly MHC-1 molecules, co-stimulatory molecules, e.g. B7, and secretory immunostimulators, e.g. cytokines, such as IL-2, IL-12, interferon and GM-CSF. The substances can be present e.g. in the form of peptides, particularly synthetic peptides. The substances can also be present in the form of expression plasmids encoding them, which can also code for HLA molecules. It is particularly favorable for the vaccination agent to also contain the cells transduced by the vector. The above explanations apply to the cells. If tumor or pre-tumor cells are concerned, it will be favorable for the cells to be inactivated.

By means of the present invention it is possible to activate the immune system against cells which are associated to papilloma viruses and sequences thereof, respectively. These cells can be tumor cells and pre-tumor cells, respectively. The activation of the immune system can be made prophylactically and in the treatment. The present invention represents a new step of treating the most severe diseases via an *in vivo* gene therapy and *ex vivo* gene therapy, respectively.

The invention is explained by the below example.

Example: Preparation of a vector coding for an HPV16 L1-E7 fusion polypeptide

The L1-ORF of a genomic HPV16 clone (cf. Kirnbauer et al., (1993), 6929-6936) was amplified by PCR reaction. For this purpose, L1-specific primers were used which have an additional BglII restriction site at the 5' end. The amplified DNA fragment was cleaved using BglII and inserted in the BamHI restriction site of the common vector pUC19. An EcoRV restriction site, followed by a translation stop codon (TAA), was introduced at position 7051 of the L1-ORF by specific mutagenesis. By this, it was achieved that the L1-ORF coded for an L1 which was lacking the last 34 amino acids.

In another PCR reaction, the part of the E7-ORF of HPV16 was amplified which codes for the first 50 amino acids of E7. The employed primers included an EcoRV restriction site at their 5' end. The amplified DNA fragment was inserted in the EcoRV restriction site of the above pUC19 vector which codes for the shortened L1. Thus, an L1-E7 fusion gene was obtained. It was inserted in the common baculovirus vector pVL1392 via XbaI/SmaI. The L1-E7 fusion gene was cleaved therefrom by NotI/SmaI and inserted in the NotI restriction site of the AAV vector pUF2 (cf. Zolotukhin et al., J. Virol. 70, (1996), 4646-4654). A vector was obtained which codes for an HPV16 L1-E7 fusion polypeptide. Viral particles of the vector were obtained according to common methods (cf. Rolling and Samulski, Molecular Biotechnology 3, (1995), 9-15).